CERTIFICATE O	F MAILING BY "EXP	RESS MAIL" (37 CFR 1.10)	Doc	ket No.
Applicant(s): Zebedee	, Suzanne, et al.	DE RO	323-1	100US D
Application No.	Filing Date FFB	0 6 2007 Examiner	Customer No.	Group Art Unit
10/677,956	10/01/2002 \ `[	7#h	20532	1648
nvention: METHOD	AND SYSTEMS FOR PRO	TRADESING RECOMBINANT VIRAL	ANTIGENS	
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10/677,956

Attorney Docket No. 323-100US-D



### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

În re	Application of:	)	Group Art Unit: 1648
	ZEBEDEE et al.	)	Examining Attorney: Zachariah Lucas
Seria	No.: 10/677,956	)	
Filed:	October 1, 2003	)	Date: February 6, 2007 Pasadena, California
For:	METHODS AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS	, ) )	

## SUBMISSION OF MARKED UP PAGES OF WANG UNITED STATES PATENT NO. 5,106,726 FILE HISTORY

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

The attached 24 pages are from the Wang Patent file history and are the same 24 pages referred to in the Supplemental Amendment filed January 31, 2007 at page 18, second paragraph. These enclosures may have been omitted in the copy of the file. Any omission is regretted.

Date: February 6, 2007

Respectfully submitted,

Registration No. 20,532

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	1		According to the present invention, a peptide		
	2	compos	ition useful for the detection of antibodies to HCV	* and	
	3		sis of MAMBH comprises a peptide selected from the		
	4		tides with the following sequences:	•	. ~
	5 6 7	(i)	Glu-Glu-Sen-Cys-Gln-Nis-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ale-Glu-Gln-Phe-Lys-Gln-Lys-Ale-Leu-Gly-Leu-Leu-Gln-Thr-Ale-Ser-Arg-Gln-Ale-Glu-Vel-Ile-Glu-Ale-Glu-Vel-Ile-Glu-Ale-Glu-Vel-Ile-Glu-Ale-Glu-Vel-Ile-Glu-Ale-Glu-Vel-Ile-Glu-Ale-Glu-Vel-Ile-Glu-Ale-Glu-Vel-Ile-Glu-Ale-Glu-		
	8	(ii)	Vel-Ile-Ala-Pro-X	(1)	•
_	9	(11)	Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X	•	
	10	(iii)	•	. (11)	
	11	(111)	Ser-Gly-Lys-Pro-Als-Ile-Ile-Pro-Asp-Arg-Glu-Val- Leu-Tyt-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser- Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu- Als-Glu-Gln-Phe-Lys-Gln-Lys-Als-Leu-Gly-Leu-X	(IIH)	-
	13 <sup>°</sup>	(iv)	Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly- Lys-Pro-Als-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr- Arg-Glu-Phe-Asp-Glu-Mat-Glu-Glu-Cys-Ser-Gln-His-	<b>,</b>	
	15		Leu-Pro-Tyr-Ile-X	(111)	ri .
•	16 17	(v)	Ser-Gly-Lys-Pro-Ala-Fle-Fle-Pro-Asp-Arg-Glu-Val- Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser- Gln-His-Leu-Pro-Tyr-Fle-Glu-Gln-Gly-Met-Met-Leu- Ala-Glu-Gln-Phe-X	. (IŸ)	•
	16	(vi)	Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-	. ()	
	19		Arg-Gln-Ala-Glu-Val-Tle-Ala-Pro-Ala-Val-Gln-Thr- Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His- Met-Trp-Asn-Phe-X	(V)	
Ð	20 21 22	(vii)	Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln- Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln- Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp- Gln-Lys-Leu-Glu-Thr-X	(VI)	
	23	(vili)	Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala- fle-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala- Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser- Arg-Gly-Asn-His-Val-Ser-Pro-X	(VII)	
	25 26 27 28	(ix) 21N3 pisw	Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg- (All-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu- Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr- Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg- Arg-X, and	(A111)	2
	29	1			
	30		- 16 -		
	1.7	distant			

```
(x)
             Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-
             Gly-X
                                                                (IX)
      wherein X is -OH or -NH_2, and analogues, segments, mixtures,
 5
      combinations, conjugates and polymers thereof.
               The amino acids in this application are abbreviated as
 7
 8
      shown herein below:
 9
10
               A- Alae alanine,
               R- Arg- arginine.
11
               D= Asp= Aspartic acid,
13
               N- Asn- siparagine,
13
               Q= Gln= glutamine,
14
               Re Glu= glutamic acid,
15
               L+ Leu- leucine,
16
               K= Lys- lysine,
17
               H- His- histidine,
18
               T= Thr= threonine,
19
               G- Gly- glycine,
20
               Y- Ile- isoleucine.
21
               F- Phe- phenylalanine,
22
                  Ser= serine,
23
                  Trp= tryptophan,
24
               Y- Tyr- tyrosine,
25
                  Val- valine,
26
               C= Cys= cysteine,
27
               P- Pro- proline
28
29
30
                                  - 17 -
```

An example of a combination is: Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Tie-Ile-Pro-Asp-Arg-Glu-2 Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Mct-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Als-Leu-Gly-Leu-Leu-Gln-Thr-Als-Ser-Arg-Gln-Als-Glu-Yal-Ils-Als-Fro-X wherein X is -OH or  $-NH_2$ . An example of a segment of Peptide II is: Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met- $\textbf{Mot-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X} \ \ \textbf{wherein} \ \ \textbf{X}$ 9 is -OH or - $\mathrm{HH}_2$  (IIF). An example of a segment of Peptide III 10 11 Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-12 13 Phn-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-fle-X wherein X is -OH or  $-NH_2$  (IIID). An example of a segment of 14 Peptide IX is Tcp-Ala-Glm-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-15 Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-16 Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-17 Gly-X (IXC). 18 The present invention also includes a highly sensitive 19 and accurate method of detecting antibodies to HCV in body 20 fluids and of diagnosing MAMBH comprises the following steps: 21 Preparing a peptide composition comprising a 2.2

peptide selected from the group having the following amino acid 23 sequences: 24

M)

25

26

27

2/3

29

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(11) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ber-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gin-Lys-Ala-Leu-Gly-Leu-X (II)

1 • 2	(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val- Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser- Glm-His-Leu-Pro-Tyr-Ile-Glu-Glm-Gly-Met-Met-Leu-	
	Ala-Glu-Gln-Phe-Lys-Gln-Lys-Als-Leu-Gly-Leu-X	(HII)
3 4	(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly- Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr- Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-	
5	Leu-Pro-Tyr-11e-X	(111)
6	(v) Ser-Gly-Lys-Pro-Als-Ile-Ile-Pro-Asp-Arg-Glu-Val- Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-	
7	Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Mat-Lau- Ala-Glu-Gln-Phe-X	(IV) : ::
. 8	(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-	
9 10	Arg-Gin-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gin-Thr- Asn-Trp-Gin-Lya-Leu-Glu-Thr-Phq-Trp-Ala-Lya-His- Met-Trp-Asn-Phe-X	; (V)
••	(vii) Glu-Gln-Gly-Net-Mat-Lau-Ala-Glu-Gln-Pha-Lya-Gln-	
11	Lys-Als-Leu-Gly-Leu-Leu-Gln-Thr-Als-Ser-Arg-Gln- Als-Glu-Val-fle-Als-Pro-Als-Val-Gln-Thr-Ass-Trp- Gln-Lys-Leu-Glu-Thr-X	(VI)
13	(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-	<b>:</b>
14	Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala- Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-	
15	Arg-Gly-Asn-His-Val-Ser-Pro-X	(VII)
16	(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-	•
	His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro- Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu- Dro-Arg-Lau-Gly-Val-Arg-Als-Thr-	16
17	Pro-Arg-Arg-Cly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr- Arg-Lya-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-	رف
18	Arg-X, and	(VIII)
19 20	(E) Gly-Arg-Arg-Glm-Pro-Ile-Pro-Lys-Vel-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ale-Gln-Pro-Gly-Tyr-Pro-Trp-	<b>L</b>
	Pro-Leu-fhp-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly- Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-	
21	Gly-Fro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu- Gly-X	(IX)
23	wherein X is -OH or -NH2, and analogues, segments, mixtu	ires,
-24	combinations, conjugates and polymers thereof; and	
25	b. Using an effective amount of the peptide	
26	composition as the antigen in an immunoassay procedure:	
27	Further, according to the present invention, t	he
28	peptides by themselves, or when coupled to a protein or	
29	polymeric carrier of home or hetero dimers or higher of	igomer#
	Bolkmetic cattlet of your or warra	
	- 19 -	
	200	

:

(13-5 and 13-6). The results were screen tested in a blood book setting.

Figure 14-1 provides a study of surum camples collected over a ten year period of time from a NANBH patient who asto-converted after receiving HCV infected blood. The committee were tested by a third EIA format designated as C constraint with Peptides IIH, V, and VIIE at 5, 3 and 2 ug/mL espectively) in comparison to two other EIA formats (designated as A and B.)

Piqure 14-2 provides another kinetic study with serum samples, kindly provided by Dr. D. Bradley of Center for diseases Control, from a chimpanzee which sero-converted after being inoculated with a well-characterized strain of MCV and contracted NANBH. These samples were tested by the HCV EIA format C, in comparison to a RIA using rDNA based HCV C-100 protein as the antigen. The ALT levels are also indicated with each bleed as a reference parameter.

Figures 15-1 and 15-2 both provide a side-by side data comparison via x-y plots with samples from hemodialysis national, kindly provided by investigators at the Japanese Hational Institute of Health. The results were obtained by using the peptide based HCV EIA Format C (coated with peptides derived from both the structural and non-structural proteins containing IIH, V and VIIIE at 5, 3, and 2 ug/mL respectively), HCV EIA Format A (coated with peptides derived from the nonstructural protein region containing IIH and V at 5 and Jug/mL respectively), and the recombinant HCV C-108 protein based EIA.

. 26

	1	by sti	mulating the production of antibodies to HCV. These		
	2	peptid	es are arranged in the following sequences:	C	7
	3	(i) ·	Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln- Gly-Met-Met-Leu-Als-Glu-Gln-Phe-Lys-Gln-Lys-Als-		
	4 5		Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu- Val-Ile-Ala-Pro-X	(1)	
•	6	(ii)	Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe- Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-		
	7		Ile-Glu-Gln-Gly-Met-Met-Leu-Als-Glu-Gln-Lwe	(II) .	
	9	(iii)	Ser-Gly-Lys-Pro-Als-Ile-Ile-Pro-Asp-Arg-Glu-Val- Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-		
•	10		Gin-His-Leu-Pro-Tyr-He-Glu-Glu-Gly-Leu-X Als-Glu-Gln-Phe-Lys-Gln-Lys-Als-Leu-Gly-Leu-X	(IIH)	1
	11	(iv)	Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly- Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr- Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-	(111)	
	12		Leu-Pro-Tyr-Ile+X	(111)	
	14	(v)	Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val- Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser- Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-	. (IV)	1
	15	(vi)	Ala-Glu-Gln-Phe-X Lys+Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser- Lys+Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-		
	16 17	(41)	Lys+Gln-Lys-Ala-Leu-Gly-Leu-Chu-Chu-Thr- Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr- Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His- Met-Trp-Asn-Phe-X	; (V)	
	18	(vii			
	19		) Glu-Gln-Gly-Met-Met-Dau-Gln-Thr-Ala-Ser-Arg-Gln- Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln- Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp- Gln-Lys-Leu-Glu-Thr-X	(VI) ·	
	20 21	(vii	i) Pro-Gly-Als-Lau-Val-Val-Gly-Val-Val-Cym-Als-Als-		
<u>.</u>	22		Val-Gin-Trp-Het-Asi-Aig-Dec 110 Arg-Gly-Asi-His-Val-Ser-Pro-X	(VII)	
] !	23 24	(ix)	His Thr-Asn-Arg-Arg-Pro-Uln-Asp-Val-Tyr-Leu-Leu-		< (Q)
	25		Gly-Gly-Gly-Gln-lie-Val-Gly-Val-Arg-Ala-Thr- Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr- Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg- Arg-X, and	(VIII)	0)
;	26 27	(z)		* *** <u></u>	P
11	28	1	Glu-Gly-Arg-Thr-Trp-Ala-Gly-Cys-Gly-Trp-Ala-Gly- Pro-Leu-ThD-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly- Pro-Leu-ThD-Gly-Asn-Glu-Gly-Gr-Arg-Pro-Ser-Trp-	12 + 1 TH A	
	29		Trp-teu-Leu-Ser-Pro-Arg-Arg-Ser-Arg-Asn-Leu- Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu- Gly-X	(1X)	
	<b>30</b>	₩ b	ermin X is -OH or -NH2 - 26 -	i Santana Marik	

HCY.

In selecting regions of the HCV protein for epitope 1 analysis, peptides in the 40mer size range with amino acid 2 sequences covering the complete HCV C-100 protein and the 3 positulated core protein were synthesized. These were tested, for their immunoreactivity with serum from a patient positively 5 diagnosed with HCV infection. Six overlapping peptides from 6 the HCV C-100 protein region designated as I, II, III, IV,  $\nu$ and VI and two adjacent peptides form the postulated core (3 protein region designated as VIII and IX were identified to have specific immunoreactivity with the positive HCV serum. 10 Another peptide VII and its fragments, C-terminal to this 11 immunodominent region, was also found to have moderate 12 immunoreactivity with a sub population of HCV positive sera. 13 See Example 12. Peptide IIH, another analogue of Peptide 11, 14 with five additional amino acids to the M-terminus has been 15 found to be highly immunogenic and contains an additional 16 epitope recognizable by antibodies in sera from patients with 17 acute phase MANBHV infection (with elevated ALT levels). The 18 amino acid sequences of the paptides are as follows: 19 20 Glu-Glu-Ber-Cys-Gln-His-Leu-Pro-Tyr-lle-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-(i) 21 Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Acg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X 22 (I) Ile-lie-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-(11)23 Asp-Glu-Mat-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-24 Gin-Lys-Ala-Leu-Gly-Leu-X (11)25 (iii) Ser-Gly-Lys-Pro-Als-Ile-Ile-Pro-Asp-Arg-Glu-Val-26 Lau-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gin-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Het-Leu-Ala-Glu-Gln-Pha-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X 27 (IIH) (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-28 Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-29 Leu-Pro-Tyr-Ile-X (111) 30

1	(v) Sui-Gly-Lyn-Pin-Aln-lle-flo-frn-Asp-Aid-Glu-Vai- hun-Tyr-Aig-Glu-fhe-Anp-Glu-Met-Glu-Glu-Cys-Ser-	
2	Gin-His-Lon-Pro-Tyr-Jio-Glu-Glu-Gly-Met-Net-Len- Ala-Glu-Glu-Fhe-X	(IV)
3	(vi) Lys-Gln-Lyx-Ala-Leu-Gly-Leu-Gln-Thr+Ala-Set- Arg-Gln-Ala-Glu-Vai-Ile-Ala-Pro-Ala-Val-Gln-Thr- Asu-Trp-Gln-Lyx-Leu-Glu-Thr-Phe-Ttp-Ala-Lyx-His- McI-Trp-Asu-Phe-X	•
5		(A)
6 7	(vii) Glu-Gln-Gly-Met-Met-Leu-Als-Glu-Glu-Phe-Lys-Glu- bys-Als-Leu-Gly-Leu-Leu-Glu-Thr-Als-Ser-Arg-Gln- Als-Glu-Val-Ile-Als-Pro-Als-Val-Glu-Thr-Asu-Trp- Glu-Lys-Leu-Glu-Thr-X	•
8	(viii) Pin-Gly-Alm-Lou-Val-Val-Gly-Val-Val-Cym-Alm-Ala-	
9	tle-Len-Arg-Arg-His-Val-Gly-Pro-Gly-Gly-Ala- Val-Gln-Tip-Met-Asn-Arg-Led+Jle-Ala-Phe-Ala-Ser- Arg-Gly-Asn-His-Val-Ser-Pro-X	•
10	(ix) Sgr-Thr-11c-Pro-Lys-Pro-Gln-Arg-Lyn-Thr-Lyn-Arg-	•
11	Acy Mil-Thr-Asn-Ary-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-	
12	Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-	. (10)
13	Arg-Lys-Thi-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg- Arg-X, and	(VI)I)
14	(x): Gly-Aig-Arg-Gln-Pro-Ilm-Pro-Lys-Vai-Arg-Arg-Eto-	
15	Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp- Pro-Leu-Chi)-Gly-Asn-Glu-Gly-Cys-Gly-Tip-Ala-Gly-	
16	TTP-Lau-Lau-Ser-Pro-Arg-Uly-Ser-Arg-Pro-Ser-Trp- Cly-Pro-Thi-Asp-Pro-Arg-Arg-Arg-Sur-Arg-Asn-Leu-	•
17	Gly-X	(IX)
18	The mix peptides !, II, III, IV, V and VI span	•
19		
20	region of 90 amino acids:	~~
21	Cys-Val-Val-Tie-Val-Gly-Arg-Val-Val-Leu-Ser-Cly-Lys-Pro- lle-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met	-Ala-lie- -Glo-Glo-
22	Cys-Sgr-Gln-His-Len-Pro-Tyr-llg-Glu-Gln-Gly-Met-Met-Len-Gln-Pro-Lys-Gln-Pro-Lys-Ala-Len-Gly-Len-Gln-Gln-Thr-Ala-Ser	-Ala-Glu- -Arg-Gln-
25	Arg-CTU-Val-lie-Ala-Pro-Ala-Val-Gla-Thr-Asa-Trp-Gla-Lyx Thr-Phe-Trp-Ala-Lys-Hix-Met-Trp-Asa-Phe	-Leu-Glu-
24		·
25	and were found to have apecific immunoreactivity with t	he
26	positive control serum. Table 1 shows the amino acid s	édnéuce,
27	of this immunodominant region of the HCV protein, and p	resents
28	the amino acid sequence of the six chemically synthesiz	eđ :
29	peptides, designated as I to VI and segments (A to H) t	hereof.
30	i	•
	- 29 <del>-</del>	•
		• •

Another two peptides (VIII and IX) apauning a region of 119 amino acids located inside the 5' terminal of the contribated HCV core protein:

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were found to have specific immunoreactivity with a syntementative panel of well-characterized HCV antibody positive sera.

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Table 7 shows the amino acid sequence of this immunodominant region of the postulated MCV core protein, and exempts the amino acid sequence of the ten chemically, whetherized populates. They were designated, as Populate VIII and IX with segments (A to D) thereof. Each of these peptides was coated at Sug/mL in a 10mM sodium bicarbonate buffer (pH 2.5) onto polystyrene microwell plates and tested in a three type 45 minute enzyme immunoussay.

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(3011634)704 MO15's BOLTHONS KIN BHY YO MOTERS LAMMERODOWNESS (BH) YO MOTERILISSILISSILISSILIS

	ATLATIC AND PARTY	주~~^~ 다르지않음 다마하다		- "A688	, , , , , , , , , , , , , , , , , , ,	**** ********************************	2888 545.45
TALL THE SECRET STREET OF SECRET SOME STREET SHEET SHEET SHEET STREET STREET SHEET STREET STR		#E: ARTOL SYLOT PETER DALOS VILOS COLLA TARE TO A MET TO	TOTAL (0.003 F 1990) 03144 (1995) 139426 (354.1) (3545) (3	144 1-4651 33-661 33-51 33-66 135-51 155-51	KALAPALL, PREEV. LAEF, DEMEE, CAGAL, PHIEG, GOOGLA, EOF	AS. FOLEY, LANG, GAMO, KLEET GO. S. FOLEY, LANG, GRING, KLEET GO. S. FOLEY, GAMO, KLEET TALK, GAMO, KLEET TALK, GAMO, KLEET GO. S. FOLEY, GAMO, KLEE	SEP "HANDY" JLETTY "OWLD" AR OF TAY OF THE SEP SEP SEP SEP SEP SEP SEP SEP SEP SE
		ភពមន្លង	4626454 <u></u>	TENOUS .	-		* * * * * * * * * * * * * * * * * * *

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underlinde erfrüßerd residues evert i --- marginel. ' --- i moderate, or L ---- ) atrong immunareactivity - 31 --- Sor-Thr-11m-Pro-Lym-Pro-Gin-Arg-Lym-Thr-Lym-Arg Him
Thr-Asn-Arg-Arg-Pro-Gin-Asp-Val-Lym-Phe-Pro-Gly-GlyGly-Gln-11m-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-ArgGly-Fro-Arg-Leu-Gly-Val-Arg-Alg-Thr-Arg-Lym-Thr-SerGly-Fro-Arg-Leu-Gly-Val-Arg-Arg-X

(VIII)

Gly-Arg-Arg-Gin-Pro-Gly-Fro-Lym-Val-Arg-Arg-Pro-GluGly-Arg-Thr-Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-LeuSer-Pro-Arg-Ang-Gly-Cym-Gly-Trp-Alg-Gly-Trp-Leu-LeuSer-Pro-Arg-Arg-Sor-Arg-Asn-Leu-Gly-X

(IXD)

Peptides XIIIE and IXD were also found to have the highest reactivity in this region.

Assays for antibodies to HCV based upon chemically synthesized peptides show several advantages over assays utilizing biologic based immunoadsorbents. The peptides can wasily be synthesized in gram quantities by using automated solid-phase methods, thus providing a reproducible antigen of high integrity with consistent yields. The presence of other antigens from biological systems precludes such reproducibility. More importantly, non-specific reactivities seen in uninfected individuals are likely to be due to the heterogeneity of the preparations used for assay. This is particularly true for assays using biologically based immunoadsorbents. In these processes, the host antigens are frequently co-puritied with the desired viral protein(s). Antibodies to these contaminating antigens are frequently found in normal individuals, thus resulting in false-positive results.

The assay of the present invention clearly minimizes such false-positive reactions as encountered in the other assay systems and, at the same time, shows a high sensitivity to truly positive sera by the substantially increased signal-to-noise ratio. This increased signal-to-noise ratio probably resulted from the purity of the immunoadsorbent. The

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1	аккау	of the present invention is also highly specific,	in that
2		ean S/C ration for HCV carriers are about 80-200 ti	
3		S/C of those of the non-infected individuals. For	
4		tentative example, see Figs. 3-1 and 3-2.	
5		The peptides useful as solid phase immunoadsorb	ante
6	tor th	on detection of antibodies to HCV were synthesized !	
7 -		ical Merrifield method of solid phase peptide synt	
8		side chain protected t-Boc-amine acids to correspon	
9		llowing amino acid sequences:	10 (1)
10			(17)
- •	(1)	Glu-Glu Ser Cya-Gln-His-Len-Pro-Tyr-11e-Glu-Gln-	・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・
11	•	Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-	
		Len-Gly-Len-Len-Gin-Thr-Ala-Ser-Arg-Gin-Ala-Glu-	
12		Val-lie-Ala-Pro-X	(1)
13	(ii)	Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-	
		Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-	
14		Ile-Glu-Gln-Gly-Met-Met-Leu-Alo-Glu-Gln-Phe-Lys-	(11)
15		Gln-Lys-Ala-Leu-Gly-Leu-X	<b>\\\\\</b>
_	(iii)	Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-	
16		Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-	•
		Gln-His-Lev-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu- Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X	(11H)
7		Wig-Gin-Cin-Lug-pla-ofu-pla-vig-men-vil-pen-v	(****/
8	(iv)	Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-	
		Lys-Pro-Ala-11e-11e-Pro-Asp-Arg-Glu-Val-Leu-Tyr-	
9		Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-	(111)
		Leu-Pro-Tyr-Ile-X	(111)
10	(v)	Ser-Gly-Lys-Pro-Ala-lle-Ile-Pro-Asp-Arg-Glu-Val-	
21		ten-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-	•
		Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-	(1V)
22		Ala-Glu-Gln-Fhe-X	(14)
2	(vi)	Lys-Gln-Lys-Ala-Lau-Gly-Leu-Leu-Gln-Thr-Ala-Ser-	
23		Arg-Gln-Aia-Glu-Val-Iis-Ala-Pro-Ala-Val-Gln-Thr-	
4		Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-	(V) ·
		Met-Trp-Asn-Phe-X	(*)
15	(vii)	Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-	
16	, , , , ,	Lvs-Ala-Leu-Glv-Leu-Leu-Gln-Thr-Ala-Sei-Arg-Gln-	
. •		Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-	/V13 / / S
<b>.7</b> `		Gln-Lys-Leu-Glu-Thr-X	(VI) - 134
8	(viii)	Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-	. <del>•</del>
. •	•	Tlw-Leu-Aru-Aru-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-	•
9		Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-	(VII)
-		Acg-Gly-Asn-His-Val-Ser-Pro-X	V****

(ix) Ser-Thi-lle-Pro-Lys-Pro-Gln-Arg-Lys-Thi-Lys-Arg-MTD-The-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-1le-Val-Gly-Gly-Val-Tyr-Len-Leu-2 Equal to VIII E 1111) Track 7 Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thi-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-1 (VIII) Arg-X, and (x) Gly-Arg-Arg-Gln-Pro-Jle-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Tro-Pro-Lau-Th-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Lau-leu-Ser-Pro-Arg-Gly-Ser-Ara-Pro-Ser-Trpo Equal To IXE Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Uly-X wherein X is -NH,.

Other analogues, segments and combinations of these peptidus may be prepared by varying the amino sold sequences either by adding, subtracting, substituting, or deleting thesized t-Boc-amino acid(s).

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Following completion of assembly of the dexired blocked peptide on the resin, the peptide-resin is treated with anhydrous hydrofluoric ecid to cleave the peptide from the resin. Punctional groups of amino acids which are blocked during synthesis by benzyl-derived blocking groups are also cleaved from the peptide simultaneously. The free peptide is then analyzed and purified by high performance liquid chiomatography (HPLC) and characterized blochemically by amino acid analysis.

Longer peptides with more than about 50 amino acids may also be prepared conveniently using well known recombinant methods. The known nucleic acids codons for each of the amino acids in the peptide may be utilized and synthetic genes encoding such peptides constructed. The synthetic gene may be inserted into vector constructs by known techniques, cloned and transfected into host cells, such as E. coli, or yeast. The secreted polypeptide may then be processed and purified according to known procedures. The peptides synthesized

with Peptide IC increases significantly, followed by a marginal increase with Peptide ID, and additional increases with Poplides IE and IF. This indicates that in the HCV Paptide I chan-Alu- Gen Gen - 1740 and Human, two clusters of smino acid residues, namely LAEOF and 5 . MiPYI, are contributing to the antigenic determinant(s) of the in'v Peptide 1. Similarly, a cluster of residues namely CHE SEC Cry for Gin His Law Prottyr we and PERCHURETY in contributing to the immunoreactivity of the HCV Crys For Gin His Law ProTyr The and 7 Positive II series: another cluster of residues namely sec-why. Pro-Aca - 160 100 Pro- Acr ary conditions to the immunoreactivity of HCV 9 Puptide III series and two clusters of residues, namely Chur and clusters of residues, namely Chur and BVIAR are contributing to the immunoreactivity by HCV 10 11 12 puptides IV and V series. As shown on the bottom of Fig. 1-1, 13 a total of six spaced clusters of amino scid residues 14 tepresenting discontinuous epitopes in this immunodominant region of the HCV protein are identified as contributing to the 15 specific HCV immunoreactivity with serum sample 1. 16 Figure 1-2 illustrates an immunoreactivity profile for 17 secum sample 2 when tested on a total of 31 overlapping 18 peptides in the HCV Paptide I, II, III, IV, V and VI series. 19 There is a clear difference between the immunoreactivity 20 profiles of serum samples 1 and 2. The immunodominant epitope, المينا بالمانية المعالية المع 21 as marked by residues EGKPAL and IIPDREV, is located towards the 2> SHOULD BE 22 H-Larminus of the region. 23 one Prolime, Figure 1-3 illustrates an immunoreactivity profile for NOT THO. 24 serum 3 when tested on the same 31 HCV peptide panel. Through THIS ERROR 25 is STILL IN 26 this extensive epitope mapping analysis, serum sample 3 was THE PRINTED found to have a mimilar immunoreactivity profile to that of the factors PATENT 27 Col 26 28 Nerum sample 2. Line 35 29

		`
	1	(f) individuals with elevated (100 1.U./L) alanine
	2	aminotransferase (ALT) enzyme activity, (n=174); (on
	3	, both IIG and liff/IIID plates)
	4	(g) individuals positive for antibodies to retroviruses
	5	HIV-1(n=100), HIV-2(n=10), HTLV-I/II(n=14); #11
	6	asymptomatic, (total n=124); (on both IIG and IIF/IIID
	7.	plates)
}	8	(h) individuals with AIDS, ARC(n=200) or ATL (n=170)
	9 10	discase, (total ne270); (on both liG and liF/liiD plates) and $N=370$
	11	(i) individuals with autoimmune disease (n=20). (on 11G
	12	plates only)
	13	(j) recombinent SOD/HCV C-100 HCV-EIA repestably reactive
	14	specimens obtained from a random donor population,
	15	(n=23). (on both IIG and IIF/IIID plates).
	16	
	17	Results obtained from groups (a) and (b) are presented
	18	in Pigs. 2-1 and 2-2 respectively (data obtained on IIG plates
,	19	only), from group (c) in Pigs. 3-1 and 3-2; from groups (d) to
	20	(i) in Fig. 4, from group (j) in Table 3 and Figs. 5 and G.
	21	In brief, as shown in Figs. 2-1 and 2-2, a comparison,
	22	by signal to cutoff ratio, between the peptide based HCV-EIA of
	23	the present invention employing pertide IIG and that of $\dot{\phi}_{ij}$
	24	recombinant SOD/HCV C-100 protein based HCV-EIA produced by
	25	Chiron/Ortho. Similar dilution titers and equal ability to
	26	identify date of sero-conversion, the two parameters indicative
	27	of each assay's sensitivity, are obtained for both assays.
	28	However, the assay according to the present invention is more
	29	sensitive and confers a higher signal to cutoff ratio to its
	2.2	nacitive specimens.

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conter of the wells. In this experiment, a P/C ratio of 20 was set as the assay cutoff value, i.e. a positive agglutination pattern had a ratio of  $\leq$ 20 and a negative pattern,>20.

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A total of 20 rDNA HCV EIA repeatably reactive specimens were tested for antibodies to HCV in the above-described HCV passive hemagglutination assay (PHA) employing Peptide IIG-BSA conjugate as the solid phase. Figure 6 provides a correlation study between the peptide based HCV PHA and the recombinant based HCV EIA by their respective P/C and s/c ratios. All samples with s/c EIA ratios higher than 3 were found to be positive with the HCV PHA test. With the exception of one, all specimens having borderline s/c ratios (between 0.9 to 2) scored as negative in this PHA test.

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#### Example\_4

Detection of Antibodies to HCV By An Agglutination Assay Utilizing As the Solid Phase Immunosorbent Gelstin Particles, Erythrocytes Of Different Animal Species, Or Latex Particles Costmd with a Mixture, of HCY Peptides

One mL thoroughly washed erythrocytes, geletin particles, or polystyrene latex particles are coated with the HCV peptide mixture, or conjugates thereof at an effective concentration. The peptide mixture, or conjugates thereof, coated calls or particles are then incubated with serially diluted serial se

#### SEAMPLE 15

2	Detection Of Antibodies To MCV By Peptide Based Enzyme-Linked
3	limmunouotbent Assny Uning Format C, Pormat D, Pormat A
4	The following four groups of specimens:
\$	(a) individuals with AIDS, ARC(n=63);
6	(b) individuals positive for ABSAq, (n=50);
7	(c) individuals positivo for antibodies to HBC
8	protein, (n=22); and
9	(d) individuals with elevated (>100 f.u./L)
10	alaning aminotransforage (ALT) ensyme activity,
11	(n=86).

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with analyzed on tepresentative HCV peptide based ElAs according to the present invention, with the plates coated either with (i) peptides IIH and V at 5 and 3 ug/mL each (Format A), (ii) peptides IIH, V and VIIIE, at 5, 3 and 2 ug/mL wach (Format C, containing both the HCV core and nonstructural poptides) or (iii) Peptides VIIIE and IXD at 2 and 2 ug/mL each (Format D, HCV core peptides only).

Results obtained from the screening of a total of 221 well-characterized clinical specimens previously categorized into four groups, from (a) to (d) using a representative lot of puptide costed plates ETAs formatted as A, C or D were plotted on histograms as shown in Figs. 12-1, 12-2 and 12-3.

Out of a total of 63 AIDS/ARC patient samples amalyzed, 46.0%, 55.6% and 50.8% of the patients were found to be HCV antibodies positive using EIA formats A. C and D respectively. Out of 50 HBSAg positive individuals, 36.0%; 42.0% and 36% of the individuals were found to also be HCV antibodies positive using EIA formats A, C and D respectively. Out of 22 HBc antibody positive individuals, 27.3%, 22.7%, and

18.2% were found to be HCV antibodies positive as detected by EIA formnis A, C and D. Out of 86 patients with an glavated ALT levels, 90.7%, 91.5% and 85.4% were found to be NCV antibodies positive by SIA formets A, C and D. The overall signal to noise rathy distribution for the HCV positive samples were found to be higher with Formats C and D which included a populida (VIIIE) from the BCV core region than Format A which only employed pentides from the HCV nonstructural region as the solid phase antigen.

Except for one HBc antibody sample where the results is borderline positive (8/cutoff ratio~1.0) with the HCV EIA' Format A, Format C incorporating peptides (IIH, V and VILE) From both the HCV structural (core) and nonstructural regions was the most sensitive. The significant improvement in sensitivity makes Format C an ideal candidate for a MCV attibody screening assay.

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#### EXAMPLE 16

Comparison Of Test Results Using The Three Peptide Bazed HCY EIA Formats (A.C. And D) On Low Risk Bandom Blood Donors

Representative 264 donor specimens obtained in a blood hank setting were tested by all three EIA formats.

The regults are shown in Figures 13-1 to 13-6. The frequency distributions of the peptide based HCV-RIA signal to cutoff ratios suggested an initial reactive rate of 1.13%, 3.0% and 3.0% with formats A, C and D respectively. The negative samples have a relative low signal to cutoff ratio in all three . assay formats( see Figures 13-1, 13-3, and 13-5). Upon repeat testing, a repeatably reactive rate of 1.13%, 1.9% and 1.9% were obtained for formats A, C and D respectively. Among the

- 71 -

corresponding EIA ratios (Table 9). Among the eleven marked 1 specimens, most showed an increased level of GOT/GPT and ware 2 associated with frequent apisodus of elevated GPT proviously. All eleven specimens scored negative by the rDNA HCV C-100 based EIA. However, these same samples reacted strongly (with O.D. N 1:5) in the peptide based HCV ElA Format C. Since peptide VIII(=VIIIE) was synthesized according to amino acid sequences selected from the conserved structural (core) protein region, its inclusion in the peptide based HCV EIA (such as format C) will be particularly suitable when testing specimens 10 From geographically distinct regions where a higher chance of 11 strain-to-strain variation among the HCV isolates may be 12 encountered. 13 It is to be understood that the above examples are 14 illustrative of the present invention and are not meant to 15 16 limit the scope thereof. 17 18

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Testing of Various formats of MCV Elas with Three Mell-Characterized Seroconversion Panels

						ELA Pacio	cho		
			777	. hSt		HCV ELL	HOW BITA	HCV ETA	<u>v</u>
1086	<u> </u>		5	32	COLOO	Format, 1	(core-cs)	(core)	
	1			1	0.01	0.093	901.0	0.105	
. Penel 1	006120	60808	0.00	£ \$	2	-0.014	0.045	0.129	
		9859876	7,7	1	90.0	-6.050	\$20.0	0.:7	
		4018088	185.0	121.0	3.64	-0.250	1.017*	1.050	
		826058	401.0	352.0	0.19	0.100	10.185	7.281	
		\$\$1109* 8\$1122	4 4	<b>4</b>	6.57	16.671	9.770	. 9.311	
						0.014	-0.058	-0.00	-
Panel 2	0.32698	480815	39.0	4 5	) C	0.443	0.058	9.101	
		840825	274.0	110.00	5 6	0.039	0.128	0.185	
		880825		2.0.7	55.4	4.057	7.8354	5.984	
		691005	429.7	172.3	8.0	5.157	7.611	5.151	
				1	╽.	-0.043	0.115	0.111	
Page 1 3 20430D	204302	_	_	? 6 i	5 2	0.041	1.607	1.1087	_
		_	D	· ·		-0.043	2.504	3.114	
		106066	143.0	2		99.	9.827	9.659	_
		880928	436.0	151.0		13.786	13.630	10.366	_
									1

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Table 9

HCV Positivity in Surum Spucimuns
Obtained from Japanese Dialysis Patienta

	TONA	Pentide	Peptide	1			1
	based	based	based	1		n: times	
ode.		HCV EIA	HCV ETA	HRSAb	GOT/GPT	during	
No.	GO AIS	Format A	Pormat C		Oct. 89	1986-1988	
	Cutoff-	Cutoff .	Cutoff .	1		when GPT	
	0.40	0.205	0.204.	1		>25 1.W./L	C 1
14	0.058	-0.001	0.005	1	2/3	- 101L	
5	0.042	0.005	0.007		9/9	ŏ	
6	0.105	-0.001	-0.003		4/4	ŏ	
7	1.837	1.462	2.312	-	3/6	2	
18:	1.797	1.637	2,398	1 - 1	20/23	2	
19.8	0.011	0.001	1.603	1	7/4	ō	
0.	0.994	0.374	2.213	1	11/9	ŏ	
i	1.823	0.425	0.874	1 .	27/16		
2	0.770	0.372	0.500		17/7	9	
3	1.712	2.101	2.234		28/32	29	
4	0.002	-0.003	0.007	1 1	11/14	ő	
54	. 0.026	0.161	2,229	1 + 1	14/23	23	
6.	0.065	0.018	2.286	1 1	20/18		
7	0.021	0.000	0.011	1 + I	16/11	1	-
À	2.347	1.917	2.182	1 . 3	26/23	6	
5 I	0.008	-0.007	0.004	1 1	7/6	ō. I	
o I	0.026	0.006	-0.002	1 1	10/8	ō	
i•	0.061	0.118	1.933	1	9/6	-	
2	2.481	2', 144	2.211	-	13/19	2	
3	0.008	-0.005	-0.005	•	11/7		
4	0.009	-0.004	-0.005	1 1	4/4	0	
5	0.009	0.000	-0.003	1 1	7/2	0	
6	2.177	1.990	2.121	1 - 1	16/12	8	
7	0.023	0.003	0.015	1 1	7/3	0	
8	0.025	-0.003	0.002	*	18/11	2	
9	0.025	-0.001	-0.006	1 1	9/5	0	
n Į	0.026	U.024	-0.003	1 1	9/3		
1	0.018	~0.003	-0.007	1 • 1	1.1/5		
2.2	0.011	-0.003	1.366	-	33/52	29	
3	2.251	1.276	2.21B	1 1	8/7	0 '	
4	0.050	0.017	0.040	1 [	10/7	0	
5	0.020	-0.007	0.017	•	14/8 .	_	
6	0.033	-0.004	0.000		9/3	0 .	
7	1.396	0.718	2.121	-	17/11	· 1	
8	0.045	0.013	-0.003		13/12		
9	0.014	0.068	0.056	1 1	10/7	0	
0	0.009	0.014	0.056	+	15/0	. 10	
3	2.047	2.214	2.235		12/9		
2	0.171	0.001	0.003	1 .	11/7	0	20.3
3	1.121	0.529	2.383	<b>+</b>	18/10		
4 [	0.113	0.066	0.002	1 1	4/3	0	
5	0.032	0.003	-0.003	1 + 1	7/5	£, ,3	
6	0.039	-0.001	-0.002		11/6		
7.	0.049	0.037	2.119	1 1	16/11		

1	TUNA	Peptide	Peptide				ſ
	based	โกษาเหน	based			n: times	
Code	HCA	HCV EIA	HCV EIA	HBSAb	GOT/GPT	during	
No.	EIY OU	Poimat A	Pormat C		Oct, 89	1986-1988	٠.
1	Cutoff.	Cutoff	Cutoff -	1		when GPT	160
	0.40	0.205	0.204			25 Jus./L	K- (47)
684	0.177	0.638	2.000		24/25	33	
69	0.027	C.007	-0.007		6/3	. 0	
70	0.031	-0.006	-0.001		16/9	Ö	
71	0.781	0.473	2.151	+	13/6	14	· ·
73	0.110	0.002	0.059		13/8	0	
73	0.043	-0.002	-0.007	-	2/3	.0	ł.
74	0.014	0.001	-0.004		2/3	0	
75	0.053	0:000	0.019		15/8		1
76	0.060	0.015	0.018		14/7	G	
77	0.011	0.001	-0.004		8/8		
7:9	0.042	0.092	0.023		3/0	0	
79	0.537	0.219	1.742		11/7		
80	2.615	1.713	2.428	+	18/16	12 .	
81	2.509	2.265	2.294	9	9/4		Ī
8.2	0:019	0.000	0.120		11/5	Q.	
8.1	0.511	1.928	2.229	-	19/11	5	
84	0.020	0.016	0.095	-	12/9		
85	0.013	-0.003	0.116		10/7	. 0	
86	0.003	-0.065	-0.006		19/5		
87	0.031.	-0.009	0.009		10/6	0	
98	0.039	0.019	0.004	j i	6/2	0	
89.	0.273	0.223	2.055	-	10/8	8	
90	0.045	0.026	-0.002	1 - 1	7/3	. 3	
7 ;	0.018	0.003	-0.002		5/8	0	
92	1.974	1.127	2,189		11/23	23	
93	0.893	1.113	2.226	· •	24/19	5	
944	0.267	0.353	2-029	-	18/12	1	
95	0.026	-0.010	0.000	1 - 1	34/73	. 0	
96*	0.021	0.002	1.599		13/30	27	
97*	0.246	0.037	1.779	1 1	15/9	0	
98	2.412	1.904	2.236	-	3/9		
			1	1 4			

#### WE CLAIM:

an amino acid sequence selected from the group consisting of: Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Als-Glu-Gln-Phe-Lys-Gln-Lys-Als-Lau-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-(1) Val-Ile-Ala-Pro-X lle-lle-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-(ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-(11) Gln-Lys-Ala-Leu-Gly-Leu-X Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-(111) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-lle-Glu-Gln-Gly-Met-Mat-Leu-(lih) Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Len-Gly-Lau-X Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-(iv)

A peptide composition comprising a peptide with

(jv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Het-Glu-Glu-Cyn-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu+Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Yal-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe+Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

(vii) Glu-Gln-Gly-Met-Met-Lau-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Set-Atg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X

(viii) Pro-Gly-Ala-Len-Val-Val-Gly-Val-Val-Cys-Ala-Alatle-Len-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Len-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-ArgAlb-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-ProGly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-LeuPro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-ThrArg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-ArgArg-X, and

(VII) (49)

(TIĮŸ)

(V)

(IV)

(x) Gly-Arg-Arg-Gln-Pro-lie-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tho-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Arg-Asn-Leu-Gly-X

(1X)

wherein X is -OH or -NH2: and

- (xi) analogues, segments, mixtures, combinations, conjugates and polymers thereof.
- 2. A peptide composition according to Claim 1 comprising a combination of Peptides I, II, III and V and having the amino acid sequence:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Pha-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Mis-Leu-Pro-Tyr-Ila-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Pha-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

wherein X is -OH or -NH2 and enalogues thereof.

- 3. A peptide composition according to Claim 1
  comprising A segment of Peptide II and having an amino acid
  meguance selected from the group consisting of:
- (i) Cys-Set-Gln-His-Leu-Pro-Tyr-lle-Glu-Gln-Gly-Met-Met-Leu-Aln-Gln-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyrlle-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Als-Leu-Gly-Leu-X;
- (iii) Leu-Tyr-Arg-Glu-Phe-Azp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Als-Glu-Gln-Phe-Lys-Gln-Lys-Als-Leu-Gly-Leu-X;
- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-1le-Glu-Gln-Gly-Met-Het-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

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